Low concentrations of F-pili were effective for adsorption (Fig. 2). Saturation was reached when about 120 μg protein of the F-pili preparation was added to the 10-ml reaction tube. In our most purified preparations as little as 1 to 2 μg gave a positive reaction. Whereas the protein nature of F-pili has not actually been established, thus far we have followed the purification of F-pili as a function of the protein concentration. Electron micrographs also show that F-pili vary greatly in length (even on the same bacterium), with sizes varying from about 0.1 μ to 8 μ. It is difficult to obtain an accurate absolute count or "titer" of F-pili since even the small fragments appear to adsorb phage. For the micrographs of Fig. 1 the phage concentration was several times higher than for the standard assay (Fig. 2).

Preparations dialyzed overnight adsorbed phage very poorly. A number of divalent metal ions including Ca^{++}, Mg^{++}, and Mn^{++} restored activity (Fig. 3). The role of the metal ion in the attachment process is not known, but is consistent with an earlier finding that Ca^{++} is required for phage development (*4*).

The filtration assay is a convenient tool for studying certain general properties of F-pili. F-pili are remarkably resistant. They retain full activity after precipitation with 0.1*N* HCl, and are unaffected by lyophilization, freezing and thawing, and incubation at *p*H 11. Upon exposure to *p*H 1.9 for 30 minutes followed by neutralization, an increase in activity was observed (Table 3).

On the other hand, heating at 70°C (Table 3) for 2 minutes resulted in about 50-percent loss of activity, whereas higher temperatures or treatment with high-frequency sound completely destroyed their ability to adsorb phage. They were sedimented by centrifugation at 100,000*g* for 30 minutes and were readily precipitated with $(NH_4)_2SO_4$ (up to 30 percent, wt to vol). By these criteria, F-pili are somewhat similar to other types of pili (*9*), although only F-pili adsorb phage.

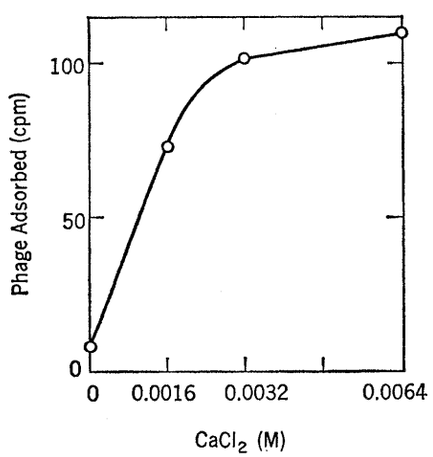

Fig. 3. Calcium-ion requirements for phage adsorption to F-pili. Incubated 5 minutes at 25°C; 0.3 ml of F-pili concentrate was used. Note optimum at 0.0032*M* $CaCl_2$.

Although RNA phages readily form complexes with F-pili, the natural role of these thin filaments in phage infection is open to some question. Phages may be detached from F-pili without loss of viability, and this indicates that their RNA was not injected into the core of the pilus. Furthermore, treatment of F-pili–phage complexes with ribonuclease neither destroyed the viability of the phage nor disrupted the complex (Table 3). Similar treatment of cell-phage complexes resulted in complete hydrolysis of the infecting RNA (Table 1). Adsorption of phage must therefore be clearly differentiated from the process of RNA injection.

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Glucose-6-Phosphatase: Reexamination of the RNA-Induced Activity in Mouse Ascites Tumor Cells

Abstract. *The glucostat method for the measurement of glucose released by glucose-6-phosphatase has been reexamined; it is accurate and sensitive enough to measure glucose-6-phosphatase activity in mouse ascites tumor cells. In seven different experiments, treatment with RNA from liver led to increases in enzyme activity varying between 125 to 200 percent. The activity of induced enzyme is optimum from* p*H 6 to 6.4.*

Ribonucleic acid is capable of inducing protein (enzyme) biosynthesis. Apparently the protein produced is related to the tissue source of the RNA (*1*). If mouse ascites tumor cells are treated with liver RNA, the cells synthesize serum albumin (*2*), glucose-6-phosphatase, tryptophan pyrrolase (*3*), catalase, and cysteine desulfurase (*4*). The acquisition of newly synthesized glucose-6-phosphatase was first shown by an increased enzyme activity of the homogenates as measured by the release of phosphate determined by a slightly modified method of Cori and Cori (*5*). This procedure was later replaced by the measurement of glucose with an improved glucostat method (reagents purchased from Worthington Company).

Imsande and Ephrussi (*6*) reported that under their conditions the glucostat method was inadequate for the assay of glucose-6-phosphatase activity in Ehrlich ascites tumor cells. Their conclusion was based on: (i) the insensitivity of the method to low concentrations of glucose; (ii) the presence of peroxidase inhibitor in ascites cell homogenates which interferes with the assay. They also suggested that the activity of ascites cell homogenates might be due to an acid phosphatase rather than to glucose phosphatase.

In view of the report of Imsande and Ephrussi, I have reexamined the adequacy of my technique for measurement of glucose-6-phosphatase activity. First the sensitivity of the glucostat method was tested with respect to the recovery of glucose added to ascites cell homogenates; this was accomplished by adding different concentrations of glucose to Nelson ascites tumor cell homogenates and then measuring the recovery of the added glucose in the incubated mixture. The optical density at 400 mμ (Table 1) of the reaction mixture (column 4) is proportional to the amount of sugar added

Table 1. Sensitivity of the glucostat method as shown by recovery of the glucose added to Nelson ascites cell homogenates. The reaction mixture contained 0.4 ml of maleate buffer (0.05*M*, *p*H 6.8), with varying amounts of glucose added; 0.5 ml of glucose-6-phosphate (G-6-P) (0.02*M*, *p*H 6.8), 0.1 ml distilled water or 0.1 ml of homogenates (1:5 water). All mixtures were incubated at 37°C for 1 hour and immediately mixed with 1 ml of ice-cold 10 percent trichloroacetic acid. They were kept at 2° to 4°C for 10 minutes. After centrifugation, 1 ml of the supernatant fluid was neutralized with 0.4 ml of a mixture containing 25 ml of potassium phosphate (0.5*M*) and 18 ml of KOH (2*N*). Then 3.5 ml of glucostat reagent (one set of reagents dissolved in 60 ml of water) were added and incubated for 1 hour at 37°C. Thereafter, one drop of 4*N* HCl was added to stabilize the color. Optical densities (O.D.) were measured at 400 mμ in a Beckman spectrophotometer.

Reaction mixture				
Glucose in 0.4 ml maleate buffer (0.05*M*, *p*H 6.8) (mμmole)	Homogenates (1:5 water) (ml)	$O.D._{400\ m\mu}$	O.D. of reaction mixture minus O.D. of homogenates plus G-6-P	Glucose recovered (%)
0	0	0		
50	0	0.050	0.050	
100	0	.105	.105	
200	0	.210	.210	
400	0	.415	.415	
800	0	.830	.830	
0	0.1	0.244		
25	.1	.267	0.023	92
50	.1	.297	.053	106
100	.1	.355	.111	106
0	0.1	0.169		
100	.1	.273	.104	99
200	.1	.383	.213	101
400	.1	.600	.431	104

(column 1). Within experimental error, recovery of the added glucose is complete (column 5). Therefore, the method is sensitive and accurate enough to measure minute amounts of glucose liberated by any acquired glucose-6-phosphatase. Hence glucose is not significantly metabolized by the homogenates, and the ascites tumor cell homogenates in the amount used here do not interfere with the glucostat assay.

The possibility that ascites tumor cell homogenates may interfere with the glucostat assay was also tested with homogenates of a different strain of ascites tumor cells. In two experiments 50 and 100 mμmole of glucose were added to the reaction mixture with and without Ehrlich ascites tumor cell homogenates (Table 2). The sample without homogenates added was taken as 100 percent glucose recovered. The recovery of glucose in the mixture was inversely proportional to the concentration of homogenates. Thus, the interference of ascites tumor cell homogenates with the assay method was directly proportional to the concentration of the homogenates. However, our determinations of the enzyme activity have been routinely made with 0.1 ml of homogenates (1:5 dilution with water). The protein content of 0.1 ml of homogenates was less than 2.0 mg, (control, 1.02 to 2.03 mg; experimental, 1.01 to 2.17 mg) at which concentration the extent of inhibition was negligible (90 percent recovery of glucose). On the other hand, the amount of homogenate protein in each determination by Imsande and Ephrussi (*6*) was much higher than the amount we used. Accordingly, interference with the glucose determination in their glucostat method is to be expected.

Our mouse colony apparently had a virus infection in 1963 (not realized until October) and again in the summer of 1964. The infection was indicated by the irregular development of the stock tumor, and frequently it failed to develop. When it eventually appeared the ratio between ascites fluid and cell volume usually deviated from the normal ratio of approximately 3:1 to an abnormal ratio of up to 30:1. Microscopic examination showed numerous cell fragments. Electron micrographs of the ascites cells revealed aggregates similar to virus particles in the cytoplasm. These aggregates were frequently larger than the nucleus of the host cell (Fig. 1). A check of our protocol book disclosed that the RNA-treated ascites cells during this period gave sporadic glucose-6-phosphatase activity and, in most cases, yielded no more enzyme activity than the controls.

For the third time, I started a fresh stock of Nelson ascites tumor cells in Princeton Swiss mice, the cells again being furnished by J. B. Nelson of Rockefeller Institute. Biosynthesis of glucose-6-phosphatase induced by liver RNA was reexamined with these new cells. The RNA treatment was carried out in vitro, or in vivo, or both. Activ-

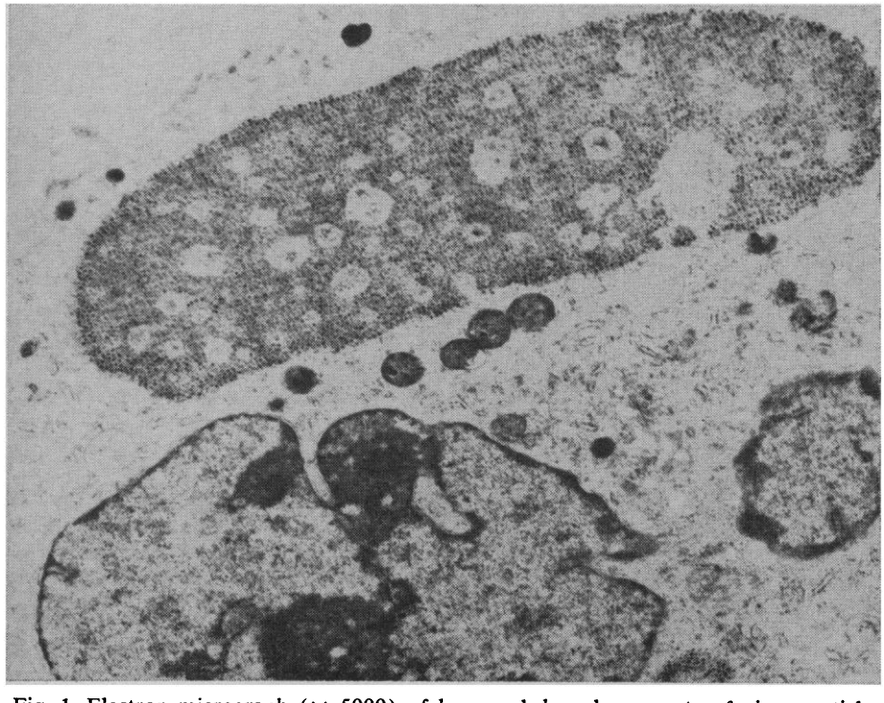

Fig. 1. Electron micrograph (× 5000) of huge rod-shaped aggregate of virus particles in a Nelson ascites tumor cell. A small cluster of virus particles can also be seen near the nucleus at the lower right.

Table 2. Effect of Ehrlich ascites cell homogenates on glucostat assay. The components of the reaction mixture were added in the order as shown in the table, and then mixed well with 1 ml of ice-cold 10 percent trichloroacetic acid. All samples were kept cold (2° to 4°C) for ten minutes. After centrifugation, 1 ml of the supernatant was neutralized and then processed as in Table 1.

Reaction mixture			O.D.* $_{400\ m\mu}$	Glucose recovered (%)
Maleate buffer (0.05*M*, *p*H 6.8) (ml)	Distilled water (ml)	Homogenates (1:5 water) (ml)		
		50 mμmole of glucose		
0.4	0.6	0	0.041	100
.4	.5	0.1	.036	90
.4	.4	.2	.027	66
.4	.1	.5	.013	32
		100 mμmole glucose		
0.5	0.5	0	0.095	100
.5	.4	0.1	.084	89
.5	.3	.2	.074	78
.5	0	.5	.052	55

* Corrected for the optical density contributed by the homogenates.

ity of the induced enzyme obtained from three methods of treatment was about the same except that the combined treatments in vitro and in vivo gave slightly more consistent results than either one alone. Figure 2*A* shows the glucose-6-phosphatase activity of the control (untreated) cells. The enzyme activity is relatively high and thus contrasts with that reported (*3*). The reason for this discrepancy is not known. The enzyme activity of the cells exposed to combined treatment with RNA both in vitro and in vivo is illustrated in Fig. 2*B*. The glucose obtained at zero time is apparently derived from free glucose associated with the substrate, glucose-6-phosphate, and with the homogenates. Its amount in ascites cells varies according to the condition of the mouse from which the ascites cells are obtained. Thus, at zero time in different experiments the ascites cell homogenates of the control series may give either higher or lower values than those of the experimental series. The enzyme activity is best measured by the linear slope of the time-course experiment (Fig. 2, *A* and *B*). The slope of both the control and the experimental series at 1 mg of protein is doubled when 2 mg of protein is used. The glucose-6-phosphatase activity of the experimental compared to the control is about 150 percent, and in six other recent experiments the activity varied between 125 and 200 percent. The specific activity of the control and

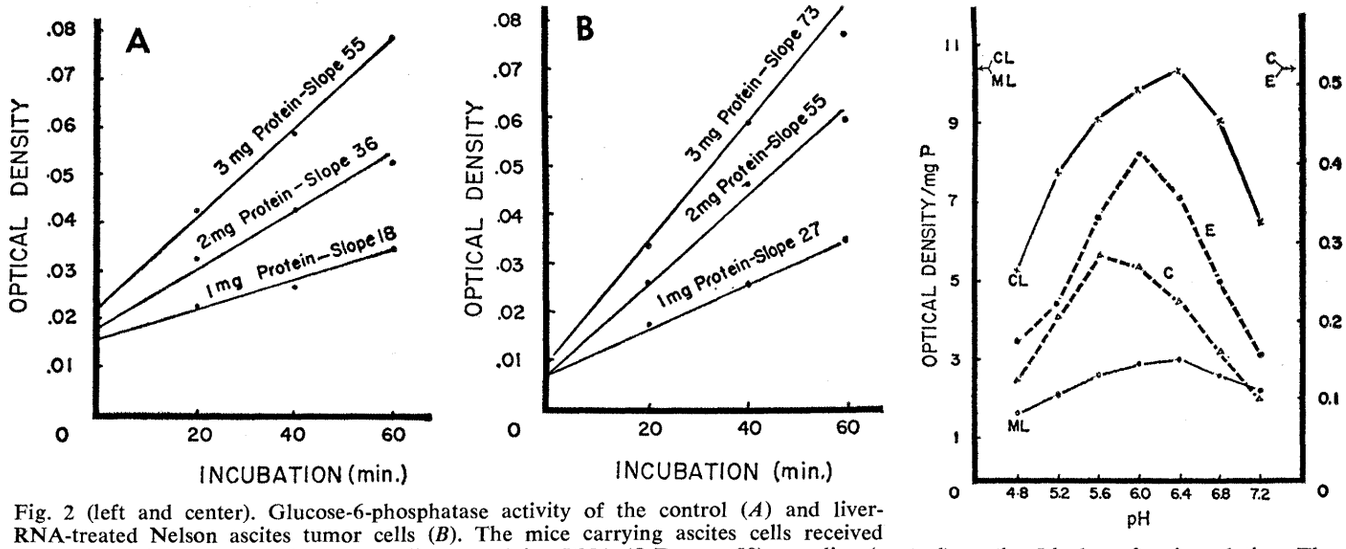

Fig. 2 (left and center). Glucose-6-phosphatase activity of the control (*A*) and liver-RNA-treated Nelson ascites tumor cells (*B*). The mice carrying ascites cells received intraperitoneal injection of 0.2 ml of saline containing RNA (O.D.$_{260\ m\mu}$ 50) or saline (control) on the 5th day after inoculation. The ascites fluid was collected on the 7th or 8th day and washed with ice-cold saline three times. Two equal volumes of packed cells were suspended separately in ten volumes of saline with or without RNA (O.D. 50 per ml), and then shaken slowly overnight (about 15 hours) in the cold (2° to 4°C). Next morning, the viability of the cells was counted (*7*). After centrifugation, the packed cells were resuspended respectively in incubation mixture with or without RNA [Krebs-Ringer phosphate buffer (*8*) with nine amino acids (*9*) or 20 amino acids, 0.5 μmole each], and enzyme induction was started in the Dubanoff incubator at 37°C for 2 hours (shaking at 90 to 100 cy/min). Viability was again checked a few minutes before completion of incubation. It should not fall below one half of the original value. Both control and RNA-treated cells were packed by centrifugation and treated with high-frequency sound in five volumes of distilled water. The incubation mixtures contained 1.5 ml of maleate buffer (0.05*M*, *p*H 6.8); 2 ml of glucose-6-phosphate (0.02*M*, *p*H 6.8); 0.5, 1.0, or 1.5 ml of Nelson ascites cell homogenates; and distilled water to a final volume of 5 ml. All mixtures were incubated at 37°C. At the start and at 20-, 40-, and 60-minute intervals, 1 ml was withdrawn from each series and immediately mixed well with 1 ml of ice-cold 10 percent TCA. The sample was kept on ice in the cold room (2° to 4°C) for 10 minutes. After centrifugation, 1 ml of the supernatant fluid was neutralized and then processed as in Table 1 except that 0.5 ml of the neutralized mixture (instead of 1.4 ml) was mixed with 4.5 ml of the glucostat reagent (dissolved in 90 ml of water). Fig. 3 (right). Effect of *p*H on the enzyme activity of calf liver (CL), mouse liver (ML), ascites tumor cell control (C), and liver-RNA-treated ascites tumor cells (E). Optical density is given per milligram of protein. The reaction mixtures contained 0.1 ml cell homogenates (ascites diluted five times, and liver 300 times); 0.4 ml buffer and 0.5 ml of glucose-6-phosphate was adjusted with acetic acid to proper *p*H. The incubation of the reaction mixtures and subsequent steps of the procedure are the same as in Table 1.

RNA-treated cell enzyme was 3.42 and 6.12 μg of glucose released, respectively, per milligram of cell homogenate protein over a period of 30 minutes (average of seven experiments).

The final question is whether the induced activity, 125 to 200 percent of the control, is due to glucose-6-phosphatase or to an acid phosphatase. The activity of glucose-6-phosphatase is optimum above *p*H 6 and near *p*H 6.8 (*5*). The activity of acid phosphatase is optimum close to *p*H 5.3 (*10*). Figure 3 shows the effect on enzyme activity of varying the *p*H. There are five points of special interest, namely: (i) The enzyme activity of calf liver is higher than that of mouse liver. (ii) The *p*H optimum of both calf and mouse liver enzyme is above 6, and maximal activity is obtained near *p*H 6.4 (iii) The activity of the control enzyme from the ascites tumor cell enzyme is optimum between *p*H 5.6 and 6. (iv) The activity of the RNA-treated enzyme from the tumor cell is optimum between *p*H 6 and 6.4. Thus RNA treatment has brought about a shift in the optimum *p*H of activity toward that of liver, with this enzymatic activity being attributable to glucose-6-phosphatase rather than to an acid phosphatase. (v) The enzyme activity of the treated cells is higher than that of the control cells. Thus, treatment of ascites cells with liver RNA leads to an increase in glucose-6-phosphatase activity.

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Cytological Basis of "Sex Ratio" in Drosophila pseudoobscura

Abstract. *Cytological investigations of both laboratory and wild "sex ratio" lines of* Drosophila pseudoobscura *reveal that, contrary to earlier reports, no extra replication of the X chromosome occurs in primary spermatocytes. Normal disjunction of the sex chromosomes at anaphase I leads to equal numbers of X-bearing and Y-bearing secondary spermatocytes. In the latter, the Y chromosome regularly shows a "degeneration" at second anaphase. The "sex ratio" effect can be explained in terms of regularly nonfunctional products of meiosis.*

In 1957, Novitski and I. Sandler (*1*) proposed that in *Drosophila melanogaster* not all products of spermatogenesis are functional. Their argument was based on the observation that from a translocation heterozygote having two pairs of unequal homologs, the probability of obtaining the various gametic types could be predicted with remarkable mathematical exactitude. Multiplication of the probabilities of recovery of any two of the individual chromosomes provided a close estimate of their joint recovery. This rule could not apply if all four products of meiosis were functional, since each homolog, being present in two of the four cells, would have a probability of recovery of 50 percent. Implicit in their proposal were two points: that the nonfunctioning of some fraction of the products was a regular phenomenon, and that this was predetermined by some geometrical property of the meiotic divisions; the latter point followed from the observation that when two homologs were present the shorter was recovered more frequently. Zimmering (*2*) repeated and extended the original observations, showing that the treatment of recovery of unequal homologs as an exercise in probability is a procedure that can be repeated for a large number of situations.

The concept of a regular class of nonfunctional spermatozoa was recently verified by Peacock and Erickson (*3*). In their analysis of the phenomenon of "segregation distortion" (*4*) these authors showed, by comparing the numbers of sperms stored by females with the numbers of progeny obtained, that only half of the sperms produced by *D. melanogaster* males are capable of fertilizing an egg. This system also permitted the conclusion that in each primary spermatocyte one pole of the first-division spindle ultimately yielded the two functional gametes, the other pole giving rise to two nonfunctional sperms. Segregation distortion was seen to result from a preferential inclusion of the segregation distorter-bearing chromosome into the "functional" pole. Peacock (*5*) subsequently showed that in instances in which there appears to be meiotic loss of chromosomes (*6*, *7*) no real cytological loss occurs; instead, the "lost" chromosomes preferentially segregate and migrate to the nonfunctional pole at the first meiotic division.

These observations cast new light on meiotic processes and provided a unified explanation of a number of meiotic abnormalities in *D. melanogaster*. On these grounds reexamination of the "sex ratio" phenomenon found in the *obscura* group of *Drosophila* seemed warranted. Analyses of "sex ratio" have been carried out by Gershenson (*7*), Sturtevant and Dobzhansky (*8*), Wallace (*9*), and others. In brief, these studies led to the following conclusions: (i) males carrying a certain X chromosome produced only daughters because all sperm were X-bearing; and (ii) all the sperm were X-bearing because at the first meiotic division the X chromosome underwent an extra replication and, at the same time, the Y chromosome degenerated. According to this scheme all four meiotic products would thus receive an X chromatid.

In the study reported here, "sex ratio" males were obtained from two different sources (*10*). All the lines we used exhibited a typical "sex ratio" effect; for example, a male of line Borrego Springs-3 yielded progeny consisting of 292 females and no males.

Cytological examinations of spermatogenesis were made for both "sex ratio" males and their wild-type sibs. In the latter males the meiotic divisions were completely regular, but in "sex ratio" males there was a striking de-